

MUNTINGIA CALABURA L.* LEAVES EXTRACT EFFECT TOWARDS INCREMENT OF NITRIC OXIDE IN *RATTUS NORVEGICUS

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ABSTRACT

Introduction: Hypertension is one of the leading health problem in Indonesia. One of the pathophysiology of hypertension is abnormality in nitric oxide production and transport. On the other side, *Muntingia calabura L.* is a plant that widely distributed in Indonesian and believed to possessed antinociceptive, antioxidant, antihypertension, and antimicrobial effect. Leaves of *Muntingia calabura L.* comprise of high phenolic compounds which is thought to increase nitric oxide production through the modulation of NOS and ROS inhibition.

Aim: To analyze the effect of extract of *Muntingia calabura L.* leaves toward increment of nitric oxide in *Rattus norvegicus*.

Method: This research is an in vivo experimental study with post-test only control group design, using nitric oxide strips test as measuring instrument. Sample of this experiment is forty *Rattus norvegicus* divided in five group which is negative control, positive control, treatment 1, treatment 2, and treatment 3. Extract of *Muntingia calabura L.* leaves with concentration 50, 100, 200 mg/mL given everyday on treatment group and measured every two days until six measurement is acquired.

Result: There is increment of NO through administration extract of *Muntingia calabura L.* leaves in *Rattus norvegicus*.

Conclusion: Highest increment of NO is obtained through administration of *Muntingia calabura L.* leaves extract at 200 mg/mL concentration.

Keywords: *Muntingia calabura L.*, nitric oxide, hypertension, nitric oxide ssynthase

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INTRODUCTION

Hypertension is a vascular disorder caused by various factors. One of the factors is the disruption of production and transport of *nitric oxide* (NO)¹. In Indonesia, hypertension is a health problem with high prevalence. According to epidemiological data on 2013, the individual analysis units show that as much as 25.8% of Indonesian people suffer from the hypertension².

Nitric oxide has a role in controlling cardiovascular homeostasis by emerging vascular functions such as vasodilation and anti-platelet aggregation. Nitric oxide is synthesized through oxidation-reduction reactions within the help of the enzyme nitric oxide synthase (NOS) and cofactors. Three types of NOS are Neuronal NOS (nNOS), Inducible NOS (iNOS), and Endothelial NOS (eNOS) 12. Nitric oxide stimulates the soluble guanylyl cyclase to form 3',5'-cyclic guanosine monophosphate (sGC/cGMP). Thus, the NO/sGC/cGMP's pathway represents the main function of NO³.

Kersen leaves (*Muntingia calabura* L.) are widely spread in Southeast Asia, including Indonesia. These leaves (*Muntingia calabura* L.) are believed to have effects as an antinociceptive, antioxidant, antihypertension, and antimicrobial⁴. Flavonoids contained in

kersen leaf (*Muntingia calabura* L.) are considered capable of stimulating NO release through NOS modulation. *Nitric oxide* causes dilatation of vascular smooth muscle thereby reducing blood pressure⁵. This study aims to analyze the NO increase in the administration of kersen leaves' extract (*Muntingia calabura* L.) to white rats (*Rattus norvegicus*).

METHOD

This research is an experimental study conducted in vivo in white rats (*Rattus norvegicus*). Moreover, the study was conducted by using the post-test only control group design method and was divided into two groups namely the control group and the treatment group.

The leaves' (*Muntingia calabura* L.) extract is made in Materia Medika Batu by the maceration method. The polar extractor used for the extract of the leaves (*Muntingia calabura* L.) is 96% ethanol. The selection of ethanol solvents is in accordance with the research of Puguh Surjowardojo and his colleagues who showed that the concentration of flavonoid compounds was higher in ethanol solvents. The leaves' (*Muntingia calabura* L.) extract is weighing on 50, 100 and 200mg were weighed using the Ohaus balance, dissolved in 2% 1 mL dimethyl sulfoxide (DMSO), and mixed until a homogeneous by using vortex.

The sample used was a white rat (*Rattus norvegicus*) obtained from the Biochemistry Laboratory of the Faculty of Medicine, Universitas Airlangga, Surabaya. The samples were selected by using a purposive sampling technique within several criterias; the male sex, by the age at 2-3 months, and a weight of 200 grams. The samples of 40 white rats (*Rattus norvegicus*) were adapted for 14 days and divided into 5 groups consisting of 8 animals in each group. The first group was the negative control group with DMSO2% intervention. The second group was a positive control group with nebivolol intervention with a dose of 0.11 mg which was calculated using the *Human Equivalent Dose* method. The third group was group P1 given intervention of kersen leaves' extract (*Muntingia calabura L.*) with a concentration of 50 mg/mL per sonde. The fourth group was a group of P2 which was given the leaves' extract (*Muntingia calabura L.*) with a concentration of 100 mg/mL per sonde. Otherwise, the fifth group, which consisted of group P3, was given extract of the leaves (*Muntingia calabura L.*) with a concentration of 200 mg/mL per sonde.

This study uses a Nitric oxide strip test that has been validated against levels of nitrite in saliva with a value of $r = 0.76^{32}$. According to Clodelter et al., regarding to the relationship between

plasma NO and salivary concentration, it was found that there was a correlation between NO_2^- in saliva and NO_3^- in plasma ($p = 0.02$)³³. In saliva, there was a reduction from NO_3^- to NO_2^- with oral *microflora*. This tool measures NOT in saliva and then compared to the color level scale. This scale has a pink to dark red color by dividing NO concentration into five ranges of values, namely depleted (0-20 $\mu\text{mol/L}$), low (21-110 $\mu\text{mol/L}$), threshold (111-220 $\mu\text{mol/L}$), target (221-435 $\mu\text{mol/L}$), and height (436-870 $\mu\text{mol/L}$). The rules to do before taking measurements; should not consume food within 10 minutes before the test, the oxide strip test should not be exposed to water, and it must be stored at 4 to 30°C. The measurements are done within every two days until six measurement is acquired.

RESULT

The data obtained from this study were processed using the *Chi-Square* significance test according to the ordinal scale. The Chi-Square test showed a value of 0,000 which means there is a significant difference between the concentration of kersen leaves extract (*Muntingia calabura L.*) on the increase in NO. Through the results of the data analysis below, the research hypothesis is accepted.

Figure 1. The Results ' Data

	1 st	2 nd	3 rd	4 th	5 th	6 th
	Measurement	Measurement	Measurement	Measurement	Measurement	Measurement
P1		Depleted	Depleted	Depleted	Depleted	Depleted
P1	Depleted	Low	Depleted	Depleted	Depleted	Depleted
P1	Depleted	Depleted	Depleted	Depleted	Depleted	Depleted
P1	Depleted	Depleted	Low	Low	Depleted	Depleted
P1	Depleted	Depleted	Depleted	Depleted	Depleted	Depleted
P1	Depleted	Depleted	Depleted	Depleted	Depleted	Depleted
P2	Low	Low	Low	Low	Low	Low
P2	Depleted	Low	Threshold	Threshold	Low	Depleted
P2	Low	Low	Low	Depleted	Depleted	Low
P2	Depleted	Threshold	Threshold	Low	Low	Low
P2	Threshold	Low	Depleted	Depleted	Depleted	Low
P2	Low	Low	Low	Depleted	Low	Low
P3	Threshold	Threshold	Threshold	Threshold	Threshold	Threshold
P3	Low	Low	Threshold	Threshold	Threshold	Low
P3	Threshold	Threshold	Low	Low	Low	Threshold
P3	Threshold	Threshold	Threshold	Threshold	Threshold	Threshold
P3	Threshold	Threshold	Threshold	Threshold	Low	Threshold
P3	Threshold	Threshold	Threshold	Threshold	Threshold	Threshold

Figure 2. The Results of Chi-Square Test

	p*
Giving <i>Muntingia calabura</i> L. *Increment of Nitric Oxide (6 days)	0,000

*Analyzed by using *Pearson Chi Square* (df=4; $\alpha=0,05$)

DISCUSSION

Previously, Z. A. Zakaria and colleagues have observed that the extract of kersen leaves (*Muntingia calabura* L.) mediates the presumable effect of

antiproliferation due to the high content of phenolic compounds⁶. According to the research by Sibi G and colleagues, regarding the phytochemical analysis of the leaves (*Muntingia calabura* L.), shows

that the leaves contain flavonoids. Moreover, the study from Clark J.L. and colleagues, regarding the efficacy of flavonoids against hypertension, said that flavonoids from 5 main subgroups namely flavones, flavonols, flavanones, and flavanols have many cardiovascular benefits such as the potential for blood pressure regulation. The mechanism of blood pressure modulation by flavonoids is thought to be through the restoration of endothelial function, affecting NO directly, indirectly, and other pathways⁷.

In a previous study conducted by Cheng-Dean Shih, an investigation of kersen leaves (*Muntingia calabura L.*) methanol extract was administrated via the intravenous route. The administration of injection of the leaves' (*Muntingia calabura L.*) extract with concentration of 10, 25, 50, 75, and 100 mg/kg causes an initial reduction and delayed onset of systemic arterial pressure. In the mice that were previously named (NOS) inhibitors, dissolved NG-nitro-L-arginine methyl ester (L-NAME, 0.325 mg/kg/min for 5 minutes) or soluble guanylate cyclase (sGC) inhibitors, 1H- [1,2, 4] oxadiazole [4,3- α] quinoxaline-1-one (ODQ, 0.2 mg/kg/min for 5 minutes). It is found that no hypotensive effect occurred.

The results of research on the increment of NO extract in kersen leaves (*Muntingia calabura L.*) toward male

white rats (*Rattus norvegicus*) obtain significant results (0,000) which means there is an increase in NO in the administration of the leaves' (*Muntingia calabura L.*) extract. Thus, their extract preparations can be developed significantly (0,000). As a matter of fact, *Muntingia calabura L.* has health benefits. These results are in accordance with Benito S and friends' research who claim that a diet rich in flavonoids increase NO production in the aorta rat. Flavonoids induce endothelium-dependent vasorelaxation in the aorta rat through increased NO⁸ production. Flavonoids can modulate NO at the cellular level by modulating NOS, namely nNOS, iNOS, and eNOS. In addition to modulating NOS, flavonoids also inhibit ROS at the cellular level resulting in an increase in NO bioavailability. According to the research conducted by Yvette et al., within the people who suffer from hypertension, there is an increment in ROS results as well as a decrement in NO bioavailability. Superoxide reacts rapidly to inactivate NO, even though the increase in superoxide is one of the mechanisms of endothelial dysfunction. Flavonoids have a direct scavenging effect on superoxide; reducing superoxide and maintaining NO levels. The increased of NO has many benefits in the vascular system, NO will stimulate the sGC/cGMP pathway causing

dilatation of vascular smooth muscle so that lowering blood pressure²⁶.

CONCLUSION

Based on the results of the research, it can be concluded that there is an increment of NO through administration extract of the leaves (*Muntingia calabura L.*) in white rats (*Rattus norvegicus*). Within the concentration of 200 mg /mL, the highest increment of NO is obtained. Its increment is linear and relates to the contribution of NO levels in the administration of the leaves extract (*Muntingia calabura L.*) with a concentration of 50, 100, and 200 mg/mL.

ACKNOWLEDGEMENT

The acknowledgement is for those who are supporting and willing for help to the thesis until manuscript:

1. Dear. Dr. dr. Endang Isbandiati, MS., Sp.FK, Silvia Sutandhio, dr., Sp.MK, M.Ked., and dr. F.X. Himawan H. Jong, M.Si as the research director.

2. Dear Mr. Heri as a laboratory assistant in Biochemical Laboratory at Universitas Airlangga.

REFERENCES

1. Hermann M1, Flammer A, L. T. Nitric Oxide in Hypertension. (2006).
2. Kemenkes.RI. Pusdatin Hipertensi. *Infodatin* 1–7 (2014). doi:10.1177/109019817400200403
3. Evora, P. R. B., Evora, P. M., Celotto, A. C., Rodrigues, A. J. & Joviliano, E. E. Cardiovascular therapeutics targets on the NO-sGC-cGMP signaling pathway: a critical overview. *Curr. Drug Targets* **13**, 1207–1214 (2012).
4. R., S. *et al.* Phytochemical Analysis of *Muntingia calabura* Extracts Possessing Anti-Microbial and Anti-Fouling Activities. *Int. J. Pharmacogn. Phytochem. Res.* **9**, (2017).
5. Ignarro, L. J. & B. F. *Nitric Oxide: Biology and Pathobiology*. (Academic Press Elsevier, 2017).
6. Zakaria, Z. A. *et al.* In vitro antiproliferative and antioxidant activities of the extracts of *Muntingia calabura* leaves. *Am. J. Chin. Med.* **39**, 183–200 (2011).
7. Clark, J. L., Zahradka, P. & Taylor, C. G. Efficacy of flavonoids in the management of high blood pressure. *Nutr. Rev.* **73**, 799–822 (2015).
8. Benito, S. *et al.* A flavonoid-rich diet increases nitric oxide production in rat aorta. *Br. J. Pharmacol.* **135**, 910–916 (2002).